Venlafaxine: in vitro inhibition of CYP2D6 dependent imipramine and desipramine metabolism; comparative studies with selected SSRIs, and effects on human hepatic CYP3A4, CYP2C9 and CYP1A2

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Aims In order to anticipate drug-interactions of potential clinical significance the ability of the novel antidepressant, venlafaxine, to inhibit CYP2D6 dependent imipramine and desipramine 2-hydroxylation was investigated in human liver microsomes. The data obtained were compared with the selective serotonin re-uptake inhibitors, fluoxetine, sertraline, fluvoxamine and paroxetine. Venlafaxine’s potential to inhibit several other major P450s was also studied (CYP3A4, CYP2D6, CYP1A2).

Methods Kᵢ values for venlafaxine, paroxetine, fluoxetine, fluvoxamine and sertraline as inhibitors of imipramine and desipramine 2-hydroxylation were determined from Dixon plots of control and inhibited rate data in human hepatic microsomal incubations. The inhibitory effect of imipramine and desipramine on liver microsomal CYP2D6 dependent venlafaxine O-demethylation was determined similarly. Venlafaxine’s IC₅₀ values for CYP3A4, CYP1A2 CYP2C9 were determined based on inhibition of probe substrate activities (testosterone 6β-hydroxylation, ethoxyresorufin O-dealkylase and tolbutamide 4-hydroxylation, respectively).

Results Fluoxetine, paroxetine, and fluvoxamine were potent inhibitors of imipramine 2-hydroxylation activity (Kᵢ values of 1.6±0.8, 3.2±0.8 and 8.0±4.3 μM, respectively, mean±s.d., n=3), while sertraline was less inhibitory (Kᵢ of 24.7±8.9 μM). Fluoxetine also markedly inhibited desipramine 2-hydroxylation with a Kᵢ of 1.3±0.5 μM. Venlafaxine was less potent an inhibitor of imipramine 2-hydroxylation (Kᵢ of 41.0±9.5 μM) than the SSRIs that were studied. Imipramine and desipramine gave marked inhibition of CYP2D6 dependent venlafaxine O-demethylase activity (Kᵢ values of 3.9±1.7 and 1.7±0.9 μM, respectively). Venlafaxine did not inhibit ethoxyresorufin O-dealkylase (CYP1A2), tolbutamide 4-hydroxylation (CYP2C9) or testosterone 6β-hydroxylase (CYP3A4) activities at concentrations of up to 1 mM.

Conclusions It is concluded that venlafaxine has a low potential to inhibit the metabolism of substrates for CYP2D6 such as imipramine and desipramine compared with several of the most widely used SSRIs, as well as the metabolism of substrates for several of the other major human hepatic P450s.

Keywords: paroxetine, fluvoxamine, sertraline, venlafaxine, fluoxetine, human liver microsomes, tricyclic antidepressants, CYP2D6

Introduction

Venlafaxine is a new antidepressant that is being used in the treatment of various depressive disorders. Unlike the serotonin selective re-uptake inhibitors (SSRIs), venlafaxine’s mechanism of pharmacological action is unique in that it involves the inhibition of the neuronal uptake of serotonin, norepinephrine and, to a lesser degree, dopamine. Venlafaxine undergoes extensive hepatic metabolism, with O-demethylation the major route of metabolism in man [1]. Over 55% of a single oral dose of venlafaxine is excreted in the urine as O-demethyvenlafaxine (ODV) and its glucuronide metabolite (ODV-glucuronide [1]). The receptor binding profile and effects on neurotransmitter uptake of ODV are similar to parent drug [2]. Venlafaxine also undergoes N-demethylation, as well as a combination of N- and O-demethylation, with N,N-dimethyvenlafaxine representing about 16% of the dose excreted in urine [1].

We have demonstrated in a previous study [3] that ODV formation is catalyzed by human hepatic CYP2D6. CYP3A4 was tentatively identified in the same study as the protein responsible for catalysing the N-demethylation pathway. As a consequence of metabolism by CYP2D6, venlafaxine competitively inhibits the activity of this enzyme. Comparative studies of the inhibitory effects of venlafaxine on CYP2D6 compared with several widely used SSRIs (fluoxetine, paroxetine, fluvoxamine and sertraline) demonstrated that the SSRIs were approximately 10–300 fold more potent CYP2D6 inhibitors (Kᵢ values, 0.065–1.8 μM) than venlafaxine (Kᵢ 20.0 μM [3]). Of the SSRIs, only paroxetine has been established to be both a substrate
and inhibitor of CYP2D6 [4, 5]. While among the SSRIs, fluoxetine is one of the most potent inhibitors of CYP2D6 [3, 6], this compound appears to be a relatively poor CYP2D6 substrate [7]. In vitro studies have also demonstrated that norfluoxetine, which is the major metabolite of fluoxetine in man [8, 9], is also a potent inhibitor of this enzyme [7, 10].

Inhibition of CYP2D6 by several of the SSRIs has been shown to have clinical consequences. For example, fluoxetine, paroxetine and sertraline significantly interact with the metabolism of desipramine, due to the effect of these agents on CYP2D6 dependent desipramine 2-hydroxylation [6, 11, 12, 13]. Elevated tricyclic antidepressant levels that occur as a result of this interaction have been associated with toxic side effects such as seizures, delirium and arrhythmias [14].

Fluoxetine also causes clinically relevant drug interactions due to inhibition of P450 enzymes other than CYP2D6, as evidenced by the finding that this agent impairs the metabolism of alprazolam, which is thought to be a substrate for CYP3A4, both in vitro and in vivo [15]. Likewise, in addition to inhibition of CYP2D6 [3], fluvoxamine is also a selective inhibitor of CYP1A2, providing the mechanistic basis for the drug interactions and resulting toxicities that have been reported when this agent is coadministered clinically with the CYP1A2 substrate theophylline [16, 17, 18].

In light of the known effects of several SSRIs on tricyclic antidepressant metabolism discussed above, the primary goal of this study was to directly assess venlafaxine’s potential to inhibit CYP2D6 dependent desipramine and imipramine 2-hydroxylation in in vitro human hepatic microsomal incubations relative to several of the most widely prescribed SSRIs (fluoxetine, paroxetine, fluvoxamine, sertraline). Inhibitory potency was assessed by measuring enzyme-inhibitor affinity constants (K_i). In addition, K_i values for imipramine and desipramine as inhibitors of CYP2D6 dependent O-demethylvenlafaxine formation were also determined, in microsomal incubations in which venlafaxine was examined as the drug substrate.

While venlafaxine is a CYP2D6 inhibitor, its effects on the catalytic activity of the other major human P450s is not known. Therefore, the second goal of this study was to further define the inhibitory potential of venlafaxine in regard to human P450s other than CYP2D6, thereby allowing us to anticipate drug-drug interactions of potential clinical relevance based on knowledge of alternate substrates for the affected P450 enzyme(s).

Methods

Chemicals

Venlafaxine (S,R-enantiomer), O-demethylvenlafaxine (ODV), N-desmethylvenlafaxine (NDV), N,O-didesmethylvenlafaxine (N,O-di DV) and WY-48818 (1-[1-(2-chlorophenyl)-2-(dimethylamino)ethyl)cyclohexanol) were synthesized at Wyeth-Ayerst Research, Princeton, NJ, USA. Fluoxetine (S,R-enantiomer) was obtained from Eli Lilly and Co. (Indianapolis, IN, USA). Sertraline (S,S-enantiomer) was obtained from Dr Edward Sellers, Addiction Research Institute, Toronto, Canada. Fluvoxamine and paroxetine (S,R-enantiomer) were available in-house. Imipramine, desipramine and mianserin were purchased from Sigma Chemicals (St Louis, MO, USA). 2-Hydroxyimipramine and 2-hydroxodesipramine were obtained from Deser and Schroeder (Madison, Wisconsin, USA). 4-Hydroxytolbutamide was from Ultrafine Chemicals (Salford, UK). 6-Hydroxytestosterone was purchased from Steraloids (Wilton, NH, USA). Ethoxyresorufin and resoru-fin was purchased from Molecular Probes (Eugene, OR, USA). All other reagents that were used were of the highest grade commercially available and were obtained from the usual sources.

Human livers

Portions of snap-frozen human liver from six donors were obtained from the International Institute for the Advancement of Medicine (IAM, Exton, PA, USA).

Preparation of microsomes and microsomal protein P450 content

Human liver microsomes were prepared by differential ultracentrifugation according to standard procedures [19]. Microsomes were stored at −80°C until use. Microsomal protein content and P450 content was determined using the procedures of Bradford [20] and Omura & Sato [21], respectively.

Enzyme and drug assays

Desipramine and imipramine 2-hydroxylation activity

Human liver microsomal incubations contained imipramine or desipramine (15 μM for IC50 studies [both substrates]) and 5–25 μM for kinetic inhibition studies [both substrates]). Microsomal protein (1 mg), MgCl2 (4 mM) and an NADPH regenerating system consisting of NADP (2 mg ml−1), glucose-6-phosphate (2 mg ml−1) and glucose-6-phosphate dehydrogenase (0.8 units ml−1), in a total incubation volume of 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.4). Incubations (25 min) were performed in a shaking water bath, and were initiated by addition of the NADPH regenerating system (60 s). Under these incubation conditions, preliminary studies demonstrated that product formation (2-hydroxydesipramine or 2-hydroxyimipramine) was linear with respect to incubation time and microsomal protein concentration. Reactions were terminated by placing the incubation tubes on ice and extracting with 3 ml of ether/dichloromethane (60:40) following addition of 5 μg of mianserin as an internal standard. The organic extract was evaporated to dryness under a stream of nitrogen gas. Dried extracts were refrigerated at 4°C before h.p.l.c. analysis (see below), which was usually on the following day.

Venlafaxine O-dealkylase activity

Incubations contained venlafaxine (30 μM for IC50 studies; 5–60 μM for kinetic inhibition studies), microsomal protein (0.6 mg), MgCl2 (4 mM) and the NADPH regenerating system described above in a total incubation volume of 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.4). Incubations (20 min) were performed in a shaker water bath, and were initiated
by addition of the NADPH regenerating system (60 μl). Preliminary studies established that O-desmethylvenlafaxine formation was linear with respect to protein concentration and incubation time under these incubation conditions. Reactions were terminated by placing tubes on ice and adding ammonium hydroxide (14.5 mM, 50 μl). Incubates were extracted with diethyl ether (5 ml, 10 min) following the addition of 1 μg of WY-45818 as an internal standard. Ether extracts were evaporated to dryness under a stream of nitrogen gas. Dried extracts were stored at 4°C before h.p.l.c. analysis (see below).

Preparation of 2-hydroxyimipramine, 2-hydroxydesipramine and O-desmethylvenlafaxine standard curves. Metabolite standard curves were generated by adding known amounts of metabolites (2-hydroxyimipramine [0.1–1.5 μmol], 2-hydroxydesipramine [0.05–1.0 μmol], O-desmethylvenlafaxine [0.02–0.5 μmol]) to a series of incubation tubes containing all of the respective reaction constituents outlined above, except the NADPH regenerating system. Tubes were taken through the entire incubation and extraction procedure followed by h.p.l.c. analysis (see below). Standard curves were generated by plotting the metabolite (2-hydroxyimipramine, 2-hydroxydesipramine, O-desmethylvenlafaxine) to internal standard (mianserin or WY-45818) peak area ratio, against the amount of added metabolite.

h.p.l.c. analysis of 2-hydroxyimipramine, 2-hydroxydesipramine and O-desmethylvenlafaxine. H.p.l.c. analyses of extracts of microsomal incubations with imipramine, desipramine and venlafaxine were performed on a Waters h.p.l.c. system consisting of a solvent pump (model 625), autosampler (model 717) and system controller (model 600E). Dry microsomal extracts were dissolved in 50–100 μl of acetonitrile/sodium phosphate buffer (pH 3.0; 50–50) immediately prior to h.p.l.c. analysis, and 35–50 μl was injected onto the chromatograph. Separations were performed on a Phenomenex Prodigy ODS2 reversed phase column (250x4.6 mm, 5 μm). Samples were eluted under isocratic conditions, using a mobile phase consisting of 0.05 M sodium phosphate buffer (pH 3.0) and acetonitrile (imipramine and desipramine, 66:33; venlafaxine, 70:30) at a flow rate of 0.6 ml min. The 2-hydroxylated metabolites of imipramine and desipramine were monitored by u.v. detection at 254 nm (Waters model 486). ODV was monitored by fluorescence detection (λ excitation and λ emission=240 and 490 nm, respectively; Waters model 470). Metabolites (2-hydroxyimipramine, 2-hydroxydesipramine and O-desmethylvenlafaxine) were identified by co-chromatography with authentic reference standards. Data were collected and peak areas integrated using Turboschrome computer software (PE Nelson, Palo Alto, CA, USA). Rates of metabolite formation were calculated from the appropriate standard curve.

Preliminary enzyme kinetic studies (imipramine/desipramine 2-hydroxylation) and IC₅₀ determinations. Preliminary enzyme kinetic studies. In light of a report describing biphasic kinetics for imipramine 2-hydroxylation in human microsomes, with CYP2D6 representing the high affinity component [22], a preliminary study was performed to confirm these findings and identify the substrate concentrations required to assay CYP2D6 activity in our experiments. In this preliminary work, the rate of imipramine 2-hydroxylation was determined over a wide substrate concentration range ([1–1000 μM]) and the data analyzed graphically by the Eadie-Hofstee method. Identical studies were also performed with desipramine as the substrate.

IC₅₀ determinations. IC₅₀ values for fluoxetine and venlafaxine were determined to obtain an initial semi-quantitative estimate of inhibitory potency in order to facilitate the selection of inhibitor concentrations to be used in subsequent quantitative enzyme kinetic studies (see below). Appropriate inhibitory concentrations of the other SSRIs were estimated from these IC₅₀ values on the basis of known relative CYP2D6 inhibitory potencies among the SSRIs. Imipramine or desipramine (both at 15 μM) were incubated with varying concentrations of venlafaxine or fluoxetine (both 0–50 μM). IC₅₀ values were also determined for imipramine and desipramine as inhibitors of venlafaxine O-demethylation. In these studies, venlafaxine (30 μM) was incubated with varying concentrations of imipramine or desipramine (both 0–500 μM). IC₅₀ values were estimated graphically from a plot of % control (uninhibited) activity against inhibitor concentration.

Enzyme kinetic inhibition studies. The rate of 2-hydroxylation of imipramine or desipramine was assessed over a range of substrate concentrations (imipramine, desipramine; both 0–25 μM) in the presence and absence of varying concentrations of venlafaxine or fluoxetine (0–80 μM and 0–5 μM, respectively). Concentrations of setraline, fluvoxamine and paroxetine used to determine the Kᵢ values of these agents as inhibitors of imipramine 2-hydroxylation were 0–75 μM, 50–50 μM and 0–50 μM, respectively. The effect of imipramine and desipramine (0–7.5 μM) on the rate of venlafaxine O-demethylation (0–30 μM) was assessed similarly. Apparent enzyme-substrate affinity constants and maximal reaction velocities (Kₑ and Vₑₘₐₓ, respectively) were calculated using an enzyme kinetics computer program (Grafit, Erithacus Software Ltd, Staines, UK). Inhibition constants (apparent Kᵢ values) were estimated graphically by the method of Dixon [23]. Mode of inhibition was determined from Cornish-Bowden plots [24].

In vitro microsomal testosterone 6β-hydroxylation, tolbutamide 4-hydroxylation and ethynylestradiol O-dealkylation activity. Human liver microsomal CYP1A2, CYP3A4 and CYP2C9 activities were determined by measuring the 7-O-deethyl- ation of ethynylestradiol to resorufin [25, 26, 27], 6β-hydroxylation of testosterone [28] and 4-hydroxylation of tolbutamide [29], respectively.

7-Ethoxycoumarin O-deethylase activity. Resorufin formation was determined by a fluorimetric method [30]. Briefly, incubation mixtures (3 ml final volume in 0.1 M potassium phosphate buffer, pH 7.6) contained an
NADPH regenerating system, ethoxyresorufin (5 μM) and human microsomal protein (250 μg). Incubations (37 °C, 5 min) were initiated by the addition of the NADPH regenerating system. Resorufin formation was quantified from the fluorescence of a known amount of resorufin (1 nmol) added at the end of the incubation.

Testosterone 6β-hydroxylase activity. Microsomal incubations contained testosterone (100 μM), an NADPH regenerating system and microsomal protein (1 mg) in a total incubation volume of 1 ml of potassium phosphate buffer (pH 7.4). Incubations (10 min at 37 °C) were initiated by the addition of NADPH. Reactions were terminated by placing tubes on ice. Following addition of an internal standard (cortisol-18, 0.5 μg), 6β-hydroxysterosterone formation was quantified following extraction by h.p.l.c. analysis according to a published procedure [31].

Tolbutamide 4-hydroxylation activity. Microsomal incubations contained tolbutamide (200 μM), an NADPH regenerating system and hepatic microsomes (1 mg) in a total incubation volume of 0.25 ml of 0.1 M potassium phosphate buffer (pH 7.4). Incubations (60 min at 37 °C), were terminated by acidification with 1 N HCl (100 μl). Following addition of chlorpropanide (0.5 μg) as an internal standard, samples were extracted with diethyl ether (3 ml, 20 min). Extracts were dried under a stream of nitrogen gas and reconstituted in h.p.l.c. mobile phase (100 μl; see below) prior to h.p.l.c. analysis. H.p.l.c. separations were performed on a C-18 reversed phase column (150 mm x 4.6 mm, 5 μm) using the same h.p.l.c. system described above for analysis of the 2-hydroxylated tricyclic antidepressant metabolites. Sample elution was isocratic using a mobile phase of water:acetonitrile:phosphoric acid (60:40:0.04) at a flow rate of 1.5 ml min⁻¹. Peaks were monitored by on-line u.v. detection at 240 nm. 4-Hydroxytolbutamide was quantified from an appropriate standard curve.

IC₅₀ determinations. Human microsomal ethoxyresorufin O-dealkylase, tolbutamide 4-hydroxylase and testosterone 6β-hydroxylase activities were determined in the presence and absence of varying concentrations of venlafaxine (10–1000 μM). IC₅₀ values were determined graphically from a plot of % of control (uninhibited) activity against venlafaxine concentration.

Results

Kinetics of imipramine and desipramine 2-hydroxylation

In preliminary experiments with pooled human liver microsomes in which the rate of 2-hydroxyimipramine formation was studied over a wide concentration range (1–1000 μM), the kinetics of imipramine 2-hydroxylation could be resolved into two components; one of high affinity, low capacity (approximate Kᵢ = 12.9 μM, Vₘₐₓ = 70.7 pmol min⁻¹ mg⁻¹) and the other of low affinity, high capacity (approximate Kᵢ = 43.4 μM, Vₘₐₓ = 103.9 pmol min⁻¹ mg⁻¹; data not shown). Over the same concentration range, the kinetics of desipramine 2-hydroxylation were monophasic (approximate Kᵢ = 43.4 μM and Vₘₐₓ = 6.1 μM and 68.0 pmol min⁻¹ mg⁻¹, respectively; data not shown).

Effect of selected SSRIs and venlafaxine on imipramine and desipramine 2-hydroxylation

The mean (n = 3 livers) Kᵢ and Vₘₐₓ values for imipramine 2-hydroxylation [22] were 20.8 μM and 50.6 pmol min⁻¹ mg⁻¹, respectively. Imipramine markedly inhibited both imipramine and desipramine 2-hydroxylation with IC₅₀ values for the inhibition of the two activities of 1.1 and 0.8 μM, respectively (Figure 1a and 1b). Over the same concentration range, venlafaxine was only moderately inhibitory (about 20% for both activities).

Quantitative enzyme kinetic inhibition studies revealed that the mean apparent Kᵢ for sertraline, fluvoxamine, paroxetine, fluoxetine and venlafaxine as inhibitors of imipramine 2-hydroxylation approximated from Dixon plots of the enzyme inhibition data was 24.7 ± 8.9 μM, 8.0 ± 4.3 μM, 3.2 ± 0.8 μM, 1.6 ± 0.8 μM and 41.0 ± 9.3 μM, respectively (n = 3, Table 1).

The mean apparent Kᵢ for fluoxetine as an inhibitor of desipramine 2-hydroxylation estimated from Dixon plots of the corresponding data for venlafaxine were curvi-linear and consequently the Kᵢ value and mode of inhibition for venlafaxine could not be accurately determined.

Inhibition of human microsomal venlafaxine O-demethylation by imipramine and desipramine

The mean Kᵢ and Vₘₐₓ values for human liver venlafaxine O-demethylation were 28.2 μM and 39.9 pmol min⁻¹ mg⁻¹, respectively. The graphically determined IC₅₀ values for imipramine and desipramine as inhibitors of venlafaxine O-demethylation were 1.6 and 0.9 μM, respectively (Figure 2).

Inhibition of ethoxyresorufin O-dealkylase (CYP1A2), testosterone 6β-hydroxylase (CYP3A4) and tolbutamide 4-hydroxylase (CYP2C9)

Venlafaxine was a poor inhibitor of ethoxyresorufin O-dealkylase, testosterone 6β-hydroxylase and tolbutamide 4-hydroxylase in human microsomal incubations (IC₅₀ > 1000 μM, Figure 3). Fluoxetine was not evaluated for its effects on these enzyme activities.

Discussion

In accord with our earlier work [3], the data presented in the current study have demonstrated that fluoxetine, fluvoxamine and paroxetine are potent inhibitors of human microsomal CYP2D6, while sertraline is less inhibitory. Also consistent with our earlier work was the finding that venlafaxine was a less potent CYP2D6 inhibitor than the SSRIs. Comparison of the Kᵢ for the inhibition of CYP2D6 dependent imipramine 2-hydroxylation by the SSRIs and venlafaxine demonstrated that they are approximately 1.5
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Figure 1 The effect of fluoxetine (open diamond) and venlafaxine (solid diamond) on the control (uninhibited) rate of human liver imipramine (a) and desipramine (b) 2-hydroxylase activity. Each point is the mean of duplicate determinations with pooled hepatic microsomes from three human livers.

Table 1 Apparent Ki values (µM) for selected SSRIs, venlafaxine, imipramine and desipramine as inhibitors of imipramine, desipramine and venlafaxine metabolism.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Sertralinea</th>
<th>Fluvoxaminea</th>
<th>Paroxetinea</th>
<th>Fluoxetineb</th>
<th>Venlafaxineb</th>
<th>Imipramineb</th>
<th>Desipramineb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipramine 2- hydroxylation</td>
<td>24.7±8.9b</td>
<td>8.0±4.3b</td>
<td>3.2±0.8b</td>
<td>1.6±0.8b</td>
<td>41.0±9.5b</td>
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<td>/</td>
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<tr>
<td>Desipramine 2- hydroxylation</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.3±0.5b</td>
<td>c</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Venlafaxine O- demethylation</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.9±1.7b</td>
<td>1.7±0.9b</td>
<td>–</td>
</tr>
</tbody>
</table>

Mode of inhibition, determined from Cornish-Bowden plots, is indicated as a competitive, b non-competitive. c Dixon plots were curvilinear, and therefore Ki values could not be determined. Data are the mean ± s.d. of three different human livers (livers 1,2,3; livers 4,5,6).

of imipramine by fluoxetine, paroxetine and fluvoxamine observed in this study, and by others [22, 32, 34], is believed to be the mechanistic basis for the drug-drug interactions that occur when imipramine or desipramine are co-administered with these SSRIs in the clinical setting [11, 12, 35, 36]. The relatively high $K_i$ for venlafaxine as an inhibitor of CYP2D6 suggests that a significant drug interaction due to the concomitant administration of venlafaxine with these tricyclic antidepressants, as well as other CYP2D6 substrates, is less likely.

Sertraline also inhibits tricyclic antidepressant metabolism in vivo, although consistent with its lesser in vitro inhibitory effects [6], the magnitude of the interaction is small compared with the corresponding effect of fluoxetine [6, 11]. In rationalizing sertraline’s lesser effect on desipramine metabolism in vivo compared with fluoxetine, Preskorn et al. [6, 11] have recently argued that this finding is due to the combination of sertraline’s lower CYP2D6 inhibitory potency, shorter half-life (making it less likely to accumulate in the plasma and at the CYP2D6 active site in the liver) and lower plasma levels relative to fluoxetine. Extending this argument to venlafaxine, the combination of venlafaxine’s lower CYP2D6 inhibitory potency compared with fluoxetine (26 fold less, this study) or sertraline (17 fold less [3], 1.5 fold less, this study), shorter plasma half-life (venlafaxine, 4.1 h [37]; fluoxetine, 2–4 days [38]; sertraline, 1 day [39]) and comparable or lower mean steady-state plasma levels at the minimum normally effective daily dose (venlafaxine, 75 mg day$^{-1}$, 21 ng ml$^{-1}$ [37]; fluoxetine, 20 mg day$^{-1}$, 85 ng ml$^{-1}$ [40]; sertraline, 50 mg day$^{-1}$, 25 ng ml$^{-1}$ [41]) suggests that venlafaxine has a lower CYP2D6 interaction potential than fluoxetine and sertraline. It is also noteworthy that norfluoxetine (the major metabolite of fluoxetine in man [8, 9]) is present in the plasma at similar levels as parent drug after multiple dosing [39]. This metabolite has a long plasma half-life (7–15 days [40]), and is equipotent in regard to CYP2D6 inhibition [7, 10]. Hence, enzyme inhibition can persist for some time after discontinuation of fluoxetine. By comparison, O-desmethylvenlafaxine has a significantly shorter half-life (10.4 h [36]) and does not appear to inhibit CYP2D6 [3]. These differing characteristics of the major metabolites of venlafaxine and fluoxetine with respect to their disposition and CYP2D6 inhibitory potency further support the prediction that venlafaxine's effects on CYP2D6 in vivo is small relative to fluoxetine.

The finding that imipramine and desipramine were inhibitors of O-desmethylvenlafaxine formation was not unexpected based on our knowledge that all three compounds are CYP2D6 substrates. Consistent with metabolism of all three agents by CYP2D6 was the finding that both imipramine and desipramine were competitive inhibitors of venlafaxine O-demethylation. The relatively high potency of inhibition of O-desmethylvenlafaxine formation as evidenced by the low apparent $K_i$ values for imipramine and desipramine (3.9 and 1.7 µM, respectively) suggests that both tricyclic compounds have the potential to inhibit venlafaxine O-demethylation in vivo.

The finding that venlafaxine does not inhibit CYP2C9 and CYP1A2 activity in vitro suggests that drug interactions between alternate substrates for these enzymes and venlafax-
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Ione may not occur. However, it should be borne in mind that in these studies we did not assess the relative affinities of the probe P450 substrates ($K_i$) relative to the respective enzyme-inhibitor affinity constant $K_J$ for venlafaxine. Nonetheless, venlafaxine’s failure to inhibit the activity of these enzymes even at very high concentrations (>1000 μM) suggests that a significant in vivo interaction between venlafaxine and alternate substrates for CYP3A4 and CYP2C9 is unlikely. The modest inhibition of CYP3A4 dependent testosterone 6β-hydroxylase activity is consistent with data from a previous study [3], in which evidence was presented involving the use of specific chemical inhibitors (tricyclopentadione, ketoconazole) that N-desmethylvenlafaxine formation is a CYP3A4 mediated process. Based on the modest inhibition of this enzyme observed at high venlafaxine concentrations (<1000 μM) in the current study we would not anticipate a marked in vivo interaction potential between venlafaxine and alternate substrates for CYP3A4.

In summary, fluoxetine, paroxetine, and fluvoxamine are potent inhibitors of CYP2D6 dependent imipramine metabolitum in in vitro human microsomal incubations. Of the SSRIs studied, sertraline was the least potent inhibitor. These data suggest that the clinical drug interactions noted when these SSRIs are coadministered with imipramine, as well as other CYP2D6 substrates, are less likely to occur with venlafaxine, which was the least potent CYP2D6 inhibitor of the compounds studied. However, high affinity CYP2D6 substrates such as imipramine or desipramine may inhibit venlafaxine metabolism leading to elevated venlafaxine levels, and decreased levels of its major metabolite O-desmethylvenlafaxine. Finally, venlafaxine was also found to be a poor inhibitor of several other major human P450s (CYP3A4, CYP2C9 and CYP1A2). Consequently, significant interactions between venlafaxine and alternate substrates for these enzymes are not anticipated.

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